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THE SENSITIVITY OF DEVELOPING HABROBRACON TO OXYGEN[1]

A. M. CLARK AND E. B. HERR, JR.[2]

Marine Biological Laboratory, Woods Hole, Mass., Brookhaven National Laboratory, Upton, N. Y., and University of Delaware, Newark, Delaware

Injury from high tensions of oxygen has been reported for a wide range of plants and animals. These have included chromosome breakage (Conger and Fairchild, 1952) and numerous non-nuclear changes (Stadie, Riggs and Haugaard, 1944). Among insects, toxicity from oxygen, as manifested by a decrease in wing beat frequency and reduced viability, has been shown for adults of *Drosophila azteca* exposed to up to ten atmospheres of oxygen (Williams and Beecher, 1944). *Drosophila melanogaster* exposed as embryos exhibited a slight lag in development, but without concomitant effects on viability (Glass and Plaine, 1952). During investigations on the parasitic wasp, *Habrobracon*, it was found that pupal development was arrested by high tensions of oxygen. Since reports of such effects are meager, it was decided to investigate this phenomenon in more detail.

Materials and Methods

The methods of rearing *Habrobracon* and of experimentation have appeared in previous publications (Clark and Mitchell, 1951). Females from stock No. 33 were crossed to males from stock No. 1. Diploid daughters were obtained from the fertilized eggs and haploid sons from unfertilized eggs. The male and female offspring were grown together in the same culture and treated at the same stage of development. All cultures were raised at 30° C.

The stages of development at which *Habrobracon* were treated and the chronological ages that coincide with these stages are shown in Figure 1. "Larva-in-cocoon" (3–4½ days) is the period from the enclosure of the larva in the cocoon to the appearance of the meconium; "prepupa" (4½–5½ days) is the stage from the egestion of the meconium to the presence of external appendages.

The wasp cultures were exposed to air, oxygen, nitrogen, and carbon dioxide in chambers about 100 cm.^{3} in volume. The gases were obtained from commercial cylinders. No attempt was made to remove impurities since previous investigators such as Conger and Fairchild (1952) found no objectionable effects from trace contaminants in commercial cylinders.

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[2] Present address: Department of Biochemistry, Cornell University, Ithaca, N. Y.

The deleterious effects were measured by determining the incidence of adults emerging from cocoons (the eclosion ratio), the occurrence of structural abnormalities among these adults and the arrest of development.

Results

Groups of individuals that ranged from the larva-in-cocoon stage to the pigmented pupal stage were exposed to one atmosphere of oxygen or of nitrogen for one hour at 30° C. Groups exposed to oxygen as larvae in cocoons or as young prepupae showed no injurious effects. Groups treated as older prepupae or as pupae were injured, white pupae (6–6½ days) being the most sensitive (Table I,

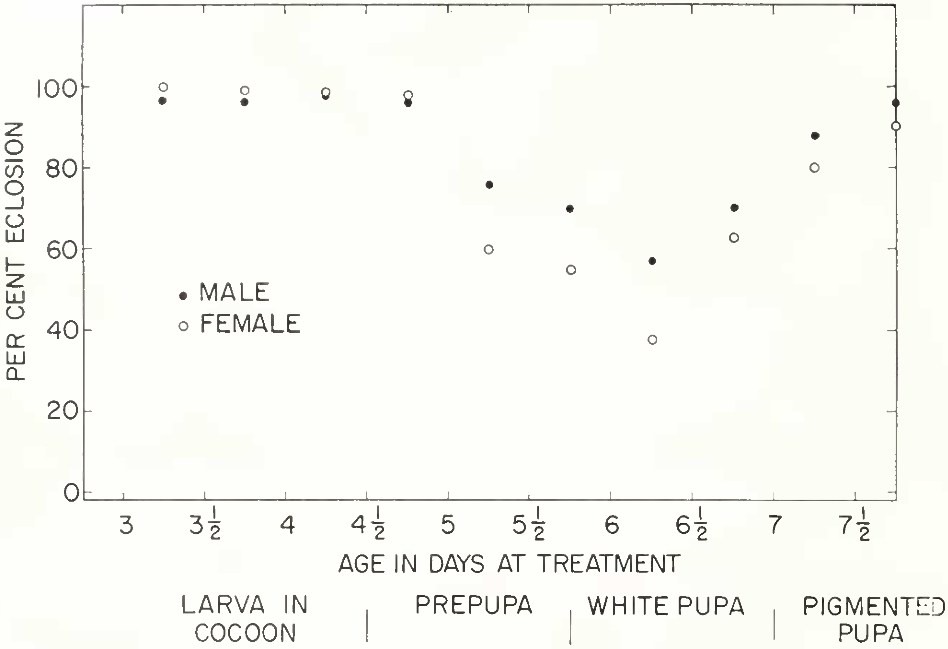

FIGURE 1. The relation of stage of development to oxygen sensitivity in Habrobracon. The deleterious effect is expressed as per cent eclosion following exposure to oxygen (100 per cent) for one hour. Data from Table I.

Fig. 1). The exposure to oxygen decreased the incidence of individuals developing to the adult stage and emerging from cocoons. Arrest of development usually occurred during the pigmented pupal stage, although such pupae remained alive for at least two weeks after eclosion of controls. An appreciable number of the adults which did emerge show wing and antennal abnormalities. The eclosion per cent for females was significantly less than for comparable males. None of the deleterious effects described for oxygen were found following exposure to nitrogen at any stage of development. The eclosion ratios for nitrogen-treated groups were not significantly different from air controls.

The preceding experiment shows that the oxygen sensitivity of *Habrobracon* varies markedly during development. Next, groups of individuals in a sensitive

TABLE I

Eclosion ratios for males and females after exposure to oxygen or nitrogen at different stages of development

Age (days) at treatment	Oxygen				Nitrogen			
	Males		Females		Males		Females	
	eclosion ratio	no. counted	eclosion ratio	no. counted	eclosion ratio	no. counted	eclosion ratio	no. counted
3–3½	.97	29	1.00	11				
3½–4	.96	182	.99	142	.90	50	.92	39
4–4½	.98	186	.98	173	.98	43	.96	49
4½–5	.96	185	.98	168	1.00	36	.92	57
5–5½	.76	286	.60	270	.96	50	1.00	33
5½–6	.70	354	.55	404	.98	58	.97	36
6–6½	.57	402	.38	452	.99	65	.98	41
6½–7	.70	371	.63	361	.93	44	.94	33
7–7½	.88	56	.80	39	.96	49	.97	35
7½–8	.96	50	.90	33	.92	53	.95	42
air controls	.96	439	.97	340				

period (white pupal stage) and during a resistant period (larva-in-cocoon stage) were exposed to air, oxygen, nitrogen or carbon dioxide at one or at two atmospheres pressure. The groups were exposed at one atmosphere for 15 minutes or at two atmospheres for 5 minutes.

Eclosion ratios following treatment of the larva-in-cocoon stage with air, oxygen, nitrogen or carbon dioxide at one or at two atmospheres indicate no deleterious effects on development or eclosion (Table II). Following exposure of white pupae to one atmosphere of nitrogen, eclosion is not significantly lower than air controls, but after one atmosphere of oxygen or of carbon dioxide eclosion is significantly lower (Table II) and after two atmospheres of oxygen or carbon dioxide there is a still greater decrease in the incidence of adults eclosing. A decrease in per cent eclosion occurs following treatment in two atmospheres of air over one atmosphere of air. Treatment in two atmospheres of nitrogen shows a non-significant decrease in eclosion for males and a small but significant decrease for females. Repetition of these experiments showed no decrease in per cent eclosion following treatment with nitrogen at two atmospheres versus one atmosphere, but did show marked differences between one and two atmospheres for air, oxygen and carbon dioxide. The difference shown for nitrogen between female pupae treated at one and at two atmospheres (Table II) is therefore spurious. Since treatment in two atmospheres of nitrogen shows no deleterious effects, it would seem that pressure itself is not responsible for the effects. The eclosion ratios following treatment in one atmosphere of oxygen or carbon dioxide are less for females than for males.

The development of white pupae exposed to two atmospheres of oxygen was arrested immediately. They remained as white pupae for one week, then started to develop pigmentation. These pupae remain alive in this arrested condition for at least three weeks after controls have emerged as adults. Observations of the arrested pupae were not continued after this period. Of comparable carbon

TABLE II

Eclosion ratios for males and females after exposure to air, oxygen, nitrogen or carbon dioxide

Gas	Sex	Larva-in-cocoon stage				White pupa stage			
		1 At.		2 At.		1 At.		2 At.	
		eclosion ratio	no. counted	eclosion ratio	no. counted	eclosion ratio	no. counted	eclosion ratio	no. counted
Air	♂	.91	54	.77	44	.90	93	.64	55
	♀	.92	64	.82	50	.90	112	.68	111
Oxygen	♂	.92	52	.85	54	.55	145	.03	77
	♀	.92	75	.86	56	.35	124	.01	74
Nitrogen	♂	.87	76	.91	45	.92	102	.82	60
	♀	.87	103	.90	40	.93	99	.75	87
Carbon dioxide	♂	.85	106	.83	40	.67	161	.23	64
	♀	.91	155	.95	59	.46	213	.09	57

dioxide-treated pupae, some continued to develop to the pigmented pupal stage before becoming arrested while others lagged for a few days as white pupae. Oxygen-treated pupae appear to be affected more than comparable pupae exposed to carbon dioxide.

Following exposure for five minutes to two atmospheres of nitrogen or to two atmospheres of oxygen, 25 white pupae were placed in each Warburg flask and oxygen uptake measured. Oxygen uptake was recorded in terms of the displacement of the Brodie's fluid, which serves sufficiently well for comparison of the effects of the gases. Upon repetitions of this experiment flasks used to measure oxygen-treated pupae were used to measure the oxygen consumption of nitrogen-treated pupae. Since the same results were obtained in all cases, errors due to slight volume differences in flasks or in the bore of the manometer are irrelevant. Neither two atmospheres of nitrogen nor one atmosphere of oxygen affected oxygen uptake. Two atmospheres of oxygen, however, resulted in an immediate and marked decrease in oxygen uptake (Fig. 2). Exposure to two atmospheres of carbon dioxide produced only a slight decrease in oxygen uptake. No decrease in oxygen uptake was observed following exposure of larvae-in-cocoons to oxygen or carbon dioxide at normal or increased pressure. The foregoing experiment shows a marked and immediate decrease in the oxygen uptake of white pupae exposed to two atmospheres of oxygen. Further work of a more quantitative nature has been planned.

DISCUSSION

Most studies have shown that the metabolic rate of immature insects is lowered by exposure to low tensions of oxygen (Wigglesworth, 1950). Effects of high oxygen tension on development have been reported by Glass and Plaine (1952) who found a slight lag in eclosion from cocoons without concomitant effects on viability for *Drosophila* exposed as embryos. The present work shows that a decrease in metabolism can be obtained by exposure to high tensions of oxygen. Pupae of *Habrobracon* are arrested in their development following such treatment and oxygen consumption is reduced. The development of pupae of the *Cecropia* silkworm is slightly delayed following exposure to 100 per cent oxygen for 21 days and slightly abnormal adults are produced (Schneiderman and Williams,

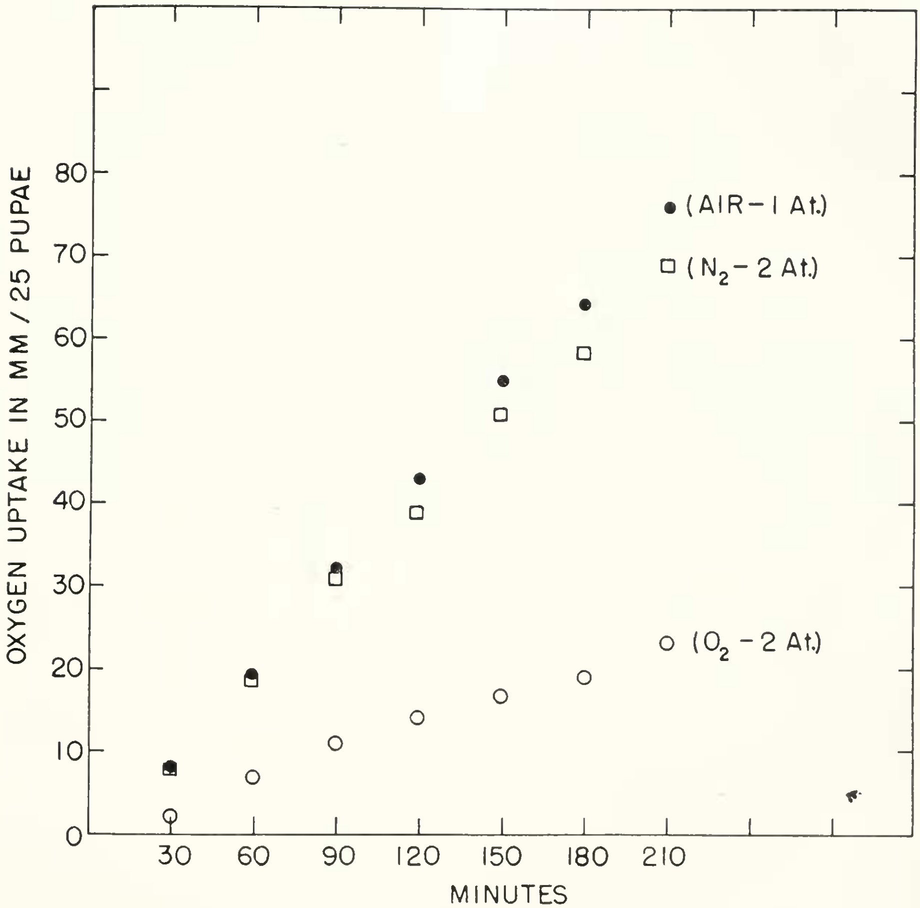

FIGURE 2. The oxygen consumption of white pupae following exposure for five minutes to two atmospheres of oxygen and of carbon dioxide. Ordinate: millimeters displacement of fluid in the arm of the manometer. Twenty-five pupae were used in each flask.

1954). In neither *Drosophila* nor *Cecropia* has treatment with oxygen at higher pressures been reported.

Work on animals as unrelated as rats, turtles and *Drosophila* adults has shown that younger animals are more resistant to injury by oxygen than older animals (Williams and Beecher, 1944). In *Habrobracon* this sensitivity is stage-dependent, since white pupae are more sensitive than the younger larvae or the older pigmented pupae. The sensitivity may be also sex-dependent since males are more resistant than females.

The oxygen-sensitive, white pupal, stage is also injured by exposure to carbon dioxide and the oxygen-resistant, larva-in-cocoon, stage is unaffected by carbon dioxide. The injury from carbon dioxide is probably not due to anoxia because the duration of exposure was short. An effect from carbon dioxide is indicated since injury from two atmospheres of carbon dioxide is greater than from one atmosphere. Williams and Beecher (1944) have shown for *Drosophila* that the toxic effects from oxygen are increased as the carbon dioxide tension is increased. The present observations can do no more than to point out the extreme sensitivity of *Habrobracon* white pupae to oxygen and to carbon dioxide. Further work is necessary to explain the mechanisms involved.

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Summary

1. Groups of *Habrobracon* were exposed during post-embryonic development to air, nitrogen, oxygen and carbon dioxide and studied with regard to development and oxygen uptake. Deleterious effects from oxygen and from carbon dioxide were observed after exposure at the white pupal stage, but not after exposure at the larva-in-cocoon stage. No deleterious effects from exposure to nitrogen were found.

2. Exposure of white pupae to one atmosphere of oxygen or carbon dioxide caused cessation of development at the pigmented pupal stage in a high proportion of the individuals. The incidence of emergence from cocoons as adults was higher for males than for females. Adults with wing and antennal abnormalities occurred. Exposure to two atmospheres of oxygen or carbon dioxide resulted in greater deleterious effects on development than did exposure to one atmosphere.

3. The oxygen uptake of unpigmented pupae exposed to two atmospheres of oxygen is markedly reduced. No such reduction was observed following treatment with two atmospheres of nitrogen or carbon dioxide or one atmosphere of oxygen.

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